#13 Decl. w/Exhibits 6.19.03

KBROL.001CP1

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Broliden et al.
Appl. No.	:	09/991,433
Filed	:	November 16, 2001
For	:	USE OF PARVOVIRUS CAPSID PARTICLES IN THE INHIBITION OF CELL PROLIFERATION AND MIGRATION
Examiner	:	Zachariah Lucas
Group Art Unit	:	1648

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF ANDERS VAHLNE M.D., PH.D.

I, Anders Vahlne, declare as follows:

1. I received my M.D. degree from the University of Gothenburg in 1973. I received my Ph.D. from the same institution in 1978. In 1994, I accepted my present position as Professor in Clinical Virology & Head of the Division of Clinical Virology at the Karolinska Institute in Stockholm, Sweden. I am a licensed physician and am affiliated with Huddinge University Hospital in Stockholm, Sweden. I am engaged in full time research in the areas of immunology and virology and have had extensive experience in these disciplines. My *curriculum vitae* is attached.

2. I have read and understand the disclosure in the application referenced above and the data presented in Exhibits A and B, which are attached to this Declaration.

3. It is evident from the application that VP2 protein and several fragments of VP2 protein of varying lengths inhibit hematopoiesis. Figures 7A-C demonstrate that LYS-C

a

endoprotease and ARG-C endoprotease cleaved VP2 protein produce a variety of VP2 protein fragments that inhibit hematopoiesis. Table 6 and Figures 8A-H demonstrate that fragments as small as 20 amino acids along the entire length of VP2 protein inhibit hematopoiesis. Further, Table 7 and Figure 9 show that fragments of VP2 protein as small as 3, 4, 6, 8, 10, 12, 16, and 21 amino acids are of a length sufficient to inhibit hematopoiesis.

4. The QQY motif is not a required element for a fragment of VP2 protein to inhibit hematopoiesis. Although the fragments of VP2 protein shown in Table 7 and Figure 9 all contain the QQY motif, the data shown in Table 6 and Figures 8A-H demonstrate that several other fragments that do not contain QQY inhibit hematopoiesis. When viewed together, the data provided in Tables 6 and 7 and Figures 8A-H and 9, provide strong evidence that fragments of VP2 protein as small as three amino acids in length, obtained from various regions of VP2 protein, inhibit hematopoiesis. Furthermore, the identification of every fragment of VP2 protein that inhibits hematopoiesis is straightforward given the disclosure in the application and the current state of protein chemistry. The sequence of VP2 protein was provided in the application in Table 6. Overlapping peptides consisting of three consecutive amino acids that span the entire length of the VP2 protein can be obtained commercially. The analysis of these tripeptides in the assays described in the application is straightforward.

5. Exhibit A shows the inhibitory effect of VP2 protein and viremic serum on hematopoietic cells of different origin. In these experiments, colony formation assays (BFU-E counts relative to a medium control; the control being 1.0) were performed according to methods described in the application. The following were analyzed:

BM(c120)7.5.99VP2 = VP2 protein incubated with human healthy bone marrow cells
FL(c150)8.3.2000VP2 = VP2 protein incubated with human fetal liver cells
FL viremic serum (c225) = B19 infectious virus particles from a viremic serum incubated with human fetal liver cells
PCV(c226) BiotrinVP2 = VP2 protein incubated with bone marrow from a patient suffering from Polycythemia vera
PCV viremic serum (c226)= B19 infectious particles from a viremic serum incubated with bone marrow from a patient suffering from Polycythemia vera
CBB19Native(Pc6) = VP1/VP2 capsid incubated with human cord blood cells

a/

FL CPV = recombinant canine parvovirus capsid protein incubated with human fetal liver cells

The data show that a 30% reduction of proliferation was observed when VP2 capsid was incubated with bone marrow obtained from a patient suffering from Polycythemia Vera (sample **PCV(c226) BiotrinVP2)**, which was significantly reduced from the medium control (medium incubated with bone marrow cells from the same PV patient) and also significantly different from the negative control (the recombinant canine parvovirus capsid protein). These experiments demonstrate that VP2 protein inhibits the hematopoiesis of hematopoietic cells obtained from an individual suffering from an hematological proliferative disorder, Polycythemia Vera.

6. Exhibit B shows the *in vivo* inhibition of hematopoietic cells in monkeys following immunization with VP2 protein. The hematocrit values of two monkeys, one of which was immunized with VP2 protein, were followed over a one month period. Monkey A received VP2 protein and monkey B did not. The data show that monkey A experienced a rapid decline in hematocrit shortly after administration of VP2 protein whereas monkey B did not. The results shown in Exhibit B establish that VP2 protein inhibits hematopoeisis *in vivo*. The data also establish that the *in vitro* results showing that VP2 protein inhibits hematopoiesis in cell culture was reasonably predictive of the outcome *in vivo*.

7. When viewed together, the data provided in Exhibits A and B provide strong evidence that VP2 protein can be administered to healthy subjects and subjects suffering from a hematological proliferative disorder such as Polycythemia Vera to inhibit hematopoiesis and thereby provide a therapeutic benefit. Furthermore, not only does the data in the application and exhibits show that VP2 protein can be used to treat hematological proliferative disorders, such as Polycythemia Vera, but the data strongly supports a finding that fragments of VP2 protein of sufficient length to inhibit hematopoiesis are also therapeutically efficacious.

8. I declare that all statements made herein are of my own knowledge, are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1.001 of Title 18 of the United States

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Code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: August 8th 2003 By: _____
Anders Vahlne, M.D., Ph.D.

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Curriculum vitae

Name Anders Gustaf Vahlne

Dat and place of birth	May 25, 1946, Lund Sweden	
Education	Licentiat of Medicine (MD), 1973 University of Gothenburg	
	Doctor of Medicine (PhD) University of Gothenburg	1978
Research Positions	Professor and head of the department of Clinical Virology, Karolinska Institutet, Stockholm	1994-
	Associate professor of Clinical Virology University of Gothenburg	1980-1993
	Visiting scientist, Scripps Clinic & Research Foundation, La Jolla CA (12 months)	1982-1983
	Research associate, Department of Clinical Virology University of Gothenburg	1970-1980
Clinical Positions	Chief physician (director) of the Laboratory of Clinical Virology, Huddinge University Hospital	1994-
	Deputy Director, Laboratory of Clinical Virology, Sahlgren's Hospital, Gothenburg	1983-1993
	Various positions as junior and senior physician at the Laboratory of Clinical Virology Sahlgren's Hospital, Gothenburg	1971-1982

Honors/Memberships

Fogarty Foundation Fellowship	1982
Swedish-American Society Award	1982

Member of the Kunkel Society
Rockefeller University

Member of the New York Academy of Sciences

Member of the Swedish Medical Society

Member of the American Association for the Advancement of Sciences (AAAS)

Tutorships

Bo Svennerholm, PhD 1981

Jan Hirsch, PhD 1983

Eva Nilheden-Thomas, PhD 1985

Per-Anders Larsson, PhD 1990

Peter Horal, PhD 1991

Tomas Bergström, PhD 1991

Ewa Hedner, PhD 1993

Agneta Samuelson, PhD 1994

External PhD examinator

at the Karolinska Institute 1983, for Lena Einhorn.

at the Karolinska Institute 1986, for Arthur Löve.

at the Karolinska Institute 1991, for Britt Åkerlind Stopner.

t the Karolinska Institute 1992, for
Matti Sällberg.

Offices etc:

Board of Directors

Tripep AB, 97-

Syntello Vaccine Development AB, Sweden, 1987-

Syntello AB, Sweden, 1986-1996

Syntello Inc./Maxim Pharmaceuticals Inc. San Diego, CA. 1992-1996.

Resistentia Pharmaceuticals AB, 1998-

Karolinska institutet, Research Committee South, 1997-

LabMedicine, Stockholm, Sweden, 1996-

Science Advisory Board of Supratek Pharma Inc., 1998-

Study section

The European Commission study section for the Biomedicine and Health Research (BIOMED 2) program.

Ad hoc referee

Proceedings for the National Academy of Scienses of the USA.

Journal of Virology

Journal of General Virology

Journal of Medical Virology

Journal of Clinical Virology

Archives of Virology

Journal of Pediatrics

Acta Patologica et Microbiologica Scandinavia

Scandinavian Journal of Infectious Diseases

Special expert

For the Professorship of Virology in Ghana, 1997.
For the Adjunct Professorship at the Karolinska Institutet of Dr. Eric Sandström, 1999.

Founder of

Syntello AB
Syntello Vaccine Development AB
Syntello Inc./Maxim Pharmaceutical Inc.
Tripep AB